Transferring HPLC methods to the Agilent 1100 Series HPLC system

Technical Note

Introduction

Today most HPLC methods must undergo a validation process, which proves that the method is suited for the desired analytical purpose. The parameters usually examined in the validation process include:

- limits of detection and quantification,
- accuracy,
- precision,
- selectivity and specificity,
- linearity,
- range, and
- ruggedness.

Limits and ranges of these parameters are influenced by the HPLC equipment in use. For example, the detection limit is strongly influenced by the noise behavior of the detector. Therefore, if a method is transferred from an existing instrument to another instrument type, for example the Agilent 1100 Series, the difference in instrument design has to be taken into consideration. In some cases the new equipment might give far better signal-to-noise ratio, or the peaks of interest elute much earlier as a result of lower system delay volume of the new HPLC instrument.

In this Technical Note we want to facilitate method transfer from an existing HPLC instrument to an Agilent 1100 Series system. Instrument characteristics of Agilent 1100 Series systems are discussed and practical examples of how these different instrument characteristics can influence the chromatographic results are given.

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Influence of HPLC module characteristics on chromatographic results

The instrument characteristics influence validation parameters mentioned earlier to a greater or lesser degree. Table 1 shows the influence of HPLC instrument components on chromatographic performance.

Consequently, before a method is transferred to the Agilent 1100 Series system the most important instrument parameters, which are responsible for differences in chromatographic performance, should be compared for each HPLC module. This can be done using table 2 where the most important instrument parameters of each Agilent 1100 Series module are shown. In the empty column the data of the existing instrument can be filled in and compared to the Agilent 1100 Series specification as a check for compliance or non-compliance.

	Pump	Degasser	Injector	Column compartment	Detector
Limit of detection and quantitation	+	+	+	+	+++++
Accuracy and precision	++++	+	+++	+	++++
Selectivity/ specificity	+++++	-	-	+	+++
Linearity/range	-	-	+++	-	++++
Ruggedness	+++++	+	+++	++	++++

+++++ = strong influence, + = low influence, - = no influence

Table 1
Influence of HPLC instrument components on chromatographic performance

	Pumps		
Parameter	**Agilent 1100 Series binary high pressure gradient pump**	**Agilent 1100 Series quaternary low pressure gradient pump**	**Exisiting pump**
System delay volume	pump <500 µl + mixer 420 µl (removable) + autosampler 300 µl (can be switched to bypass)	range: 809 to 1306 µl	
	Degasser		
Parameter	**Agilent 1100 Series degasser**		**Existing degassing device**
Degassing system	online vacuum degasser		
	Autosampler		
Parameter	**Agilent 1100 Series autosampler**		**Existing autosampler**
Injection volume range	0.1 to 100 µl up to 1500 µl with multiple draw optional 0.1 to 900 µl up to 1800 µl with multiple draw		
Precision	typically <0.5 % RSD for peak areas from 5 to 100 µl typically <1 % RSD for peak areas from 1 to 5 µl typically <0.5 % RSD for peak areas from 10 to 900 µl with optional 900-µl metering device		
Carry over	typically 0.1 % without needle wash typically <0.5 % with needle wash		
	Detectors		
Parameter	**Agilent 1100 Series diode-array detector**	**Agilent 1100 Series variable wavelength detector**	**Exisiting UV detector**
Short term noise	± 1 x 10^{-5} AU at 254 nm ± 1 x 10^{-5} AU at 750 nm	± 0.75 x 10^{-5} AU at 254 nm	
Wavelength range	190 to 950 nm in steps of 1 nm	190 to 600 nm in steps of 1 nm	
Bandwidth	selectable	typically 6.5 nm for the whole wavelength range	
Optical slit width programmable	1, 2, 4, 8, 16 nm	not applicable	
Diode width	< 1 nm	not applicable	
Flow cells	standard: 10 mm path length 13-µl cell volume semi-micro: 6-mm path length 5-µl cell volume micro/high pressure: 6 mm path length 1.7-µl cell volume	standard: 10 mm pathl ength 14-µl cell volume micro: 5-mm path length 1-µl cell volume high pressure: 10-mm path length 14-µl cell volume	

Table 2
Most important instrument parameters in chromatography

Key parameters for smooth method transfer

The most important instrument parameters for a smooth method transfer are:

- delay volume, and
- UV detector parameters.

The following sections give recommendations on how to adapt a Agilent 1100 Series system to transfer these two parameters from an existing method to an Agilent 1100 Series system.

Delay volume

The overall system delay volume is influenced by the delay volume of the pump, installed mixers and injector (see table 2). This delay volume determines the time which is needed for gradient changes to reach the column. Different delay volumes give different elution times for the same gradient profile. As shown in figure 1, the influence is less significant for flow rates greater than 1 ml/min. The system delay volume becomes significantly important if low flow rates less than 0.5 ml/min with gradient analysis are used. Increasing the delay volume results in a remarkable increase in retention times and equilibration times after the run, as shown in figure 2.

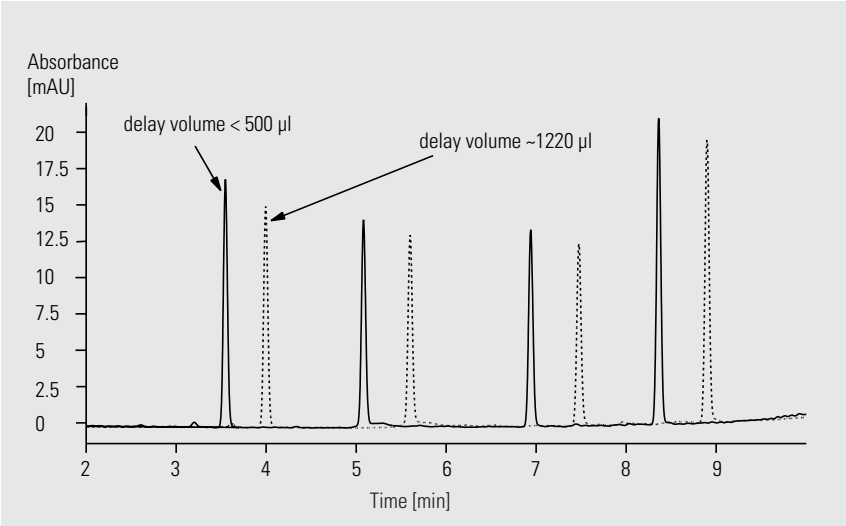

Figure 1
Influence of different delay volumes at a flow rate of 1.5 ml/min

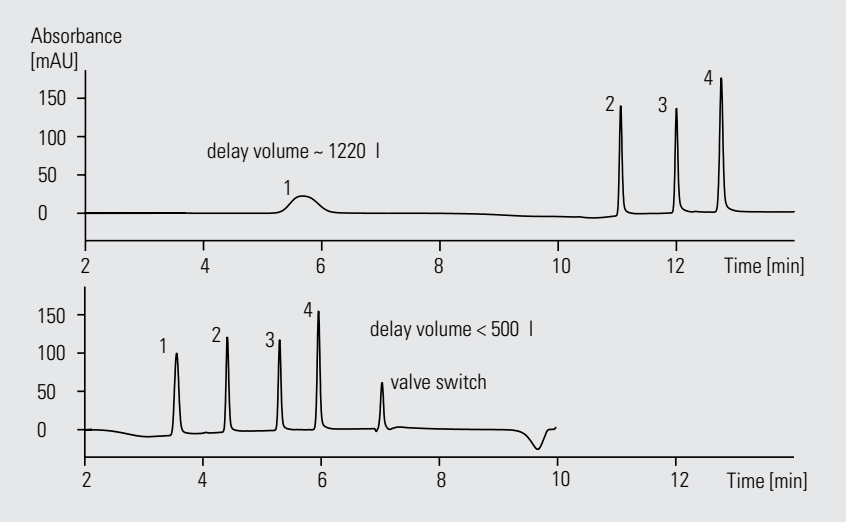

Figure 2
Influence of different delay volumes at a flow rate of 0.1 ml/min

Minimizing the influence of differences in delay volume

In the following example, four compounds were analyzed on an HPLC system with a system delay volume of 2300 µl. The method was transferred to the Agilent 1100 Series system using the binary pump with mixer. The system delay volume in this case was about 920 µl. All other parameters such as column, flow rate, gradient, column compartment temperature and detection wavelength remained the same. To obtain similar elution times the delay volume was added to the Agilent 1100 Series binary pump. A sample loop with an internal volume of 1.3 ml was installed between the pump and the autosampler, as shown in figure 3.

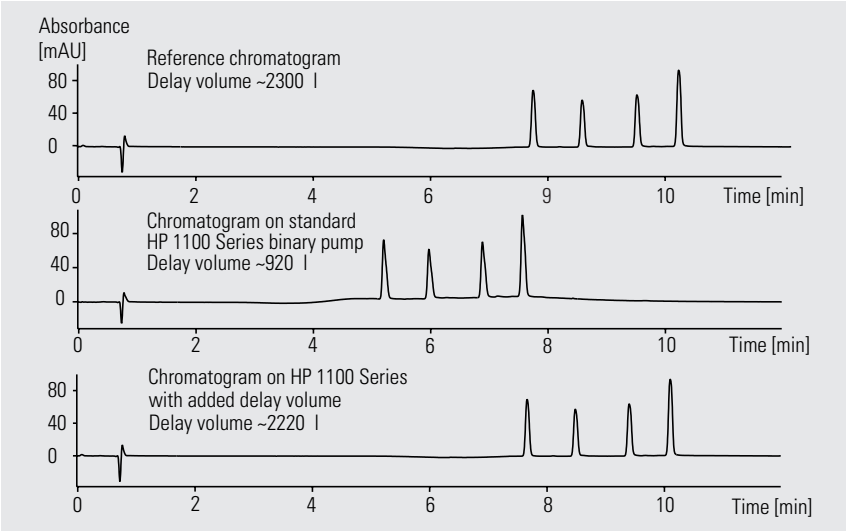

Figure 3
Minimizing the influence of delay volume on retention times by adding defined volume

Parameter	Existing HPLC instrument low pressure gradient system, system delay volume: 2300 µl	Agilent 1100 Series instrument with binary pump and added delay volume high pressure gradient system, system delay volume: 2220 µl
Sample	Isocratic standard: dimethylphthalate, diethylphthalate, biphenyl, o-terphenyl	same
Column	2.1 mm x 100 ODS Hypersil, 5 µm	same
Injection volume	1µl	same
Oven temperature	40 °C	same
Solvents	A = water = 10 % B = acetonitrile = 10 %	same same
Flow rate	400 µl/min	same
Gradient	at 5 min 95 % B	same
Detection	VWD 245/8 nm, 10 mm path length, 13 µl flow cell	DAD 254/8 nm, ref. 360/100 nm 10 mm path length, 13-µl flow cell or VWD can be used
Degassing	online vacuum degassing	same
Additional pump parameters for the Agilent 1100 Series binary pump		compressibility for A = 47 compressibility for B = 115 stroke A = 20 µl stroke B = 20 µl

UV detector parameters

For smooth method transfer, first select a detector cell with the same path length. The Agilent 1100 Series diode array detector has programmable optical slits which can be selected from the screen. No mechanical exchange is necessary, and the same slit width should be selected. The Agilent 1100 Series has a dual lamp design with a deuterium and tungsten lamp in series for optimum light intensity over a wavelength range of 190 to 950 nm. If the existing UV detector has a different design, the tungsten lamp should be switched off. All other detector settings such as detection wavelength, data rate, and so on, should remain the same.

Minimizing the influence of detection wavelength bandwidth on chromatographic results

Variable and single wavelength UV detectors have a fixed bandwidth for all possible detection wavelengths. When using diode array systems the bandwidth can be set independently for each selected detection wavelength. For a smooth method transfer it is therefore recommended to use the same bandwidth on both instruments for the selected detection wavelength.

Figure 4 gives an example of how different bandwidths influence peak heights.

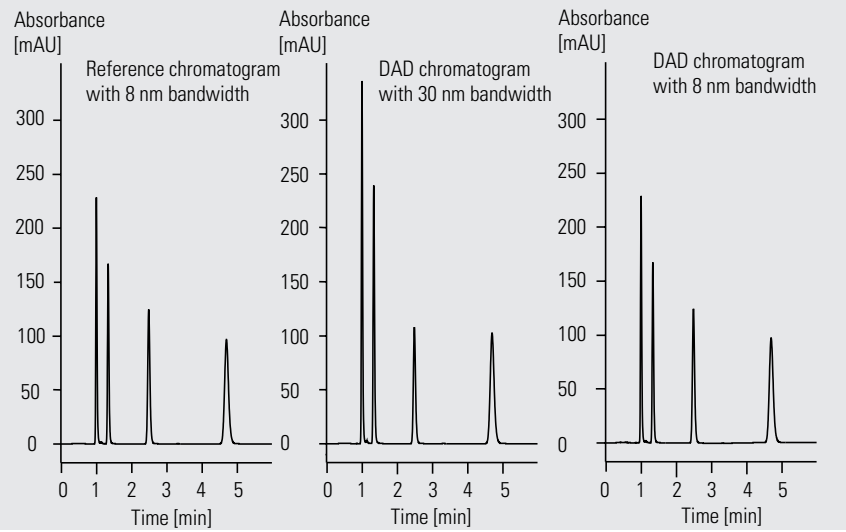

Figure 4
Influence of different bandwidths for the detection wavelength on relative peak heights

Parameter	Existing HPLC instrument low pressure gradient system	Agilent 1100 Series instrument high pressure gradient system
Sample	Isocratic standard: dimethylphthalate, diethylphthalate, biphenyl, o-terphenyl	same
Column	4 x 125 mm Hypersil ODS, 5 µm	same
Injection volume	1 µl	same
Oven temperature	40 °C	same
Solvents	A = water = 35 % B= acetonitrile = 65 %	same
Flow rate	1.5 ml/min	same
Detection	VWD 245/6.5 nm, 10 mm path length, 14 µl flow cell	DAD 254/30 nm or 8 nm , ref. 360/100 nm 10 mm path length, 13-µl flow cell
Degassing	online vacuum degassing	same
Additional pump parameters for the Agilent 1100 Series Series binary pump	-	compressibility for A = 46 compressibility for B = 115 stroke A = auto stroke B = auto

Recommendations to improve chromatographic performance with the Agilent 1100 Series HPLC systems

Carry-over

Peak area precision can deteriorate when non-reproducible sample carry-over occurs. Therefore, a sampling mode which avoids carry-over should be used, for example, a needle wash in pure solvent between two injections.

Minimizing the influence of carry-over

Two standard HPLC systems were compared with a completly different autosampler and injector design. One design was based on conventional glass syringes, which can be used at low pressures. This system did not have a needle wash option. The metering device of the Agilent 1100 Series injector has a stainless steel body and sapphire plunger. This design can be used at pressures up to 400 bar and can be flushed permanently while being in the high-pressure flow path.

The effect of needle wash between injections is shown in figure 5.

Temperature stability of column compartement

If the the column compartment temperature varies with the ambient temperature, it will influence precision of retention times, especially if the column compartment temperature is selected to be near ambient. In this case active cooling of the column compartment is needed. This can be done with either an external cooling device or a column thermostate based on peltier design which allows ambient and subambient operation with high temperature stability.

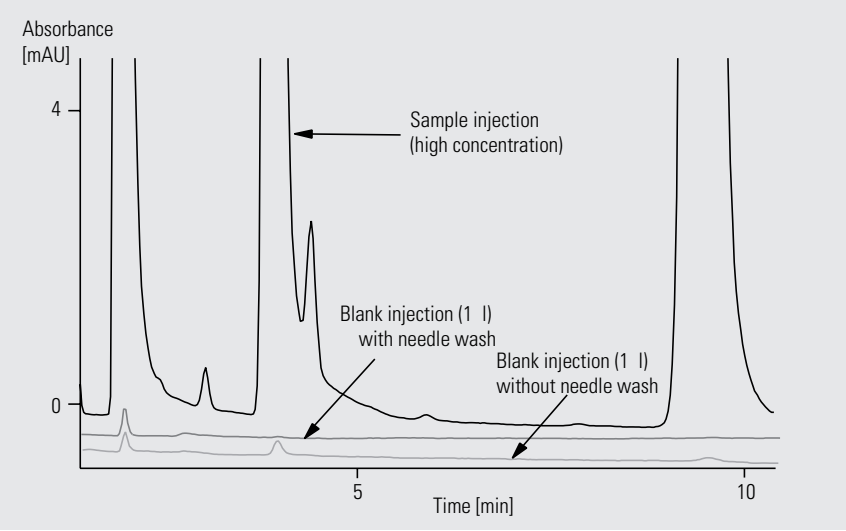

Figure 5
Influence of carry over on chromatographic performance

Parameter	Existing HPLC instrument low pressure gradient system	Agilent 1100 Series instrument high pressure gradient system
Sample	Isocratic standard: 0.01 m caffeine primi-, done, phenacetin, 1-µl injection volume	same
Column	125 x 4 mm Hypersil ODS, 5 µm	same
Injection volume	1 µl, no needle wash	same, but with needle wash
Oven temperature	30 °C	same
Solvents	A = water = 85 % B= acetonitrile = 15 %	same same
Flow rate	1.5 ml/min	same
Gradient	at 5 min 95 % B	same
Detection	DAD 220/10 nm, 10 mm path length, 13-µl flow cell	same
Degassing	online vacuum degassing	same
Additional pump parameters for the Agilent 1100 Series Series quaternary pump	-	compressibility: 100 stroke: auto

Minimizing influence of column compartment temperature fluctuations on chromatographic results

In this example we analyzed paraquat and diquat at 20 °C on a conventional instrument with a temperature range of 10 degrees above ambient up to 60 °C and on the Agilent 1100 Series with a temperature range from 10 degrees below ambient up to 80°C. Figure 6 shows that precision for retention times was significantly better using the design with active cooling. The room temperature fluctuated during the runs from 22.6 °C to 25.4 °C. A 30 minute wait time was programmed between the runs.

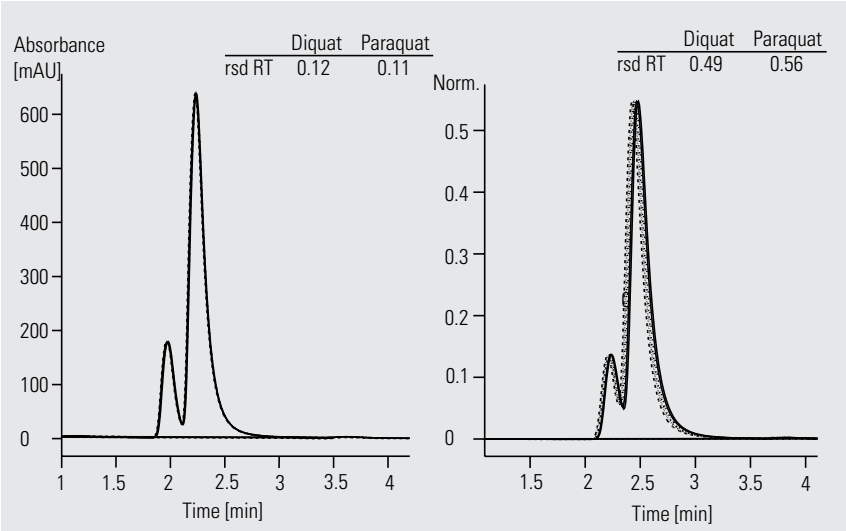

Figure 6
Analysis of paraquat and diquat at 20 °C on two different HPLC systems, with and without active cooling

Parameter	Existing HPLC instrument low pressure gradient system	Agilent 1100 Series instrument high pressure gradient system
Sample	paraquat, diquat,	same
Column	100 x 2.1 mm Hypersil ODS, 5 µm	same
Injection volume	1 µl	same
Oven temperature	20 °C	same
Solvents	A = water + 0.5 g/l hexanesulfonic acid + 3.5 ml triethylamin, pH = 2.5 with phosphoric acid	same
Flow rate	0.4 ml/min	same
Detection	VWD 256 nm	same
Degassing	online vacuum degassing	same
Additional pump - parameters for the Agilent 1100 Series quaternary pump		compressibility: 47 stroke: auto

Conclusion

Instrument characteristics significantly influence the range and limits of validation parameters. When transferring a method from an existing HPLC system to an Agilent 1100 Series system, the main instrument parameters should be comparable or set up such that they are comparable. The appearance of the chromatogram and the chromatographic results can then be compared.

Some differences and improvements in terms of chromatographic performance have to be considered. This is due to:

- highly precise gradient formation even for rapid gradients and/or extreme low flow rates using the binary pump,
- very efficient degassing using the vacuum degasser,
- precise injection over a wide injection volume range,
- low carry-over,
- highly stable column compartment temperatures from subambient to 80 °C,
- low noise behavior of the VWD and DAD, and
- spectral information from 190 to 950 nm with the DAD.

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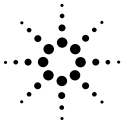

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